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Biocontrol with *Trichoderma* species for the management of postharvest crown rot of banana

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Summary. *Lasiodiplodia theobromae* and *Colletotrichum musae* cause the postharvest crown rot disease complex of banana. *In vitro* experiments evaluated the effect of twelve isolates of *Trichoderma* spp. from the soil of organic banana orchards ('native isolates') and eight isolates of *Trichoderma* spp. from culture collections ('introduced isolates') on the two pathogens. The native and introduced *Trichoderma* spp. had varied antagonistic effects against the two pathogens. Eight *Trichoderma* spp. isolates effective in the *in vitro* assays were evaluated singly on fruits both at room temperature and in cold storage. Single antagonists did not satisfactorily control crown rot on the fruits as compared with the fungicide carbendazim. However, two isolates of *T. viride,* one of *T. harzianum* and one of *T. koningii* performed well when applied singly, and these were selected for evaluation in isolate mixtures. There was very little antagonism between these isolates. Of 11 two-way, three-way and four-way mixtures of these isolates, the four-way and a three-way mixtures reduced crown rot incidence, both at room temperature and in cold storage, giving better control than carbendazim. The study identified consortia of compatible *Trichoderma* antagonists with superior biocontrol potential for the management of the postharvest crown rot complex of banana.

Key words: *Lasiodiplodia theobromae*, *Colletotrichum musae,* antagonist mixtures.

Introduction

Poststharvest losses of fruits are mainly due to fungal infection, physical injury and physiological disorders. Of the postharvest diseases that affect banana, crown rot caused by a complex of fungal pathogens (*Lasiodiplodia theobromae* [Pat.] Griffon and Maubl. and *Colletotrichum musae* [Berk and Curtis] Arx.) is a major problem in all banana growing regions (Sepiah *et al.,* 1990). This disease decreases the quality of bananas, with aesthetic damage and drop of fingers. A high incidence of crown rot can cause premature ripening during transit (Slabaugh and Grove, 1982). The disease has been reported to be a major problem for exported Cavendish group banana fruits (Jones, 2000).

Normally, fungicides are the prime means of controlling postharvest diseases (Eckert *et al.,* 1994). Crown rot of banana is controlled commercially by submerging clusters of bananas in solutions of thiabendazole (TBZ), imazalil or benomyl (Sepiah and Nik Mohd, 1987; Krauss *et al.,* 1998; Aked *et al.,* 2001). The use of synthetic chemicals to control postharvest rots and deterioration has been limited due to their potential carcinogenicity, teratogenicity, environmental pollution, effects on food and other side-effects

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on humans (Unnikrishnan and Nath, 2002; Mari *et al.,* 2003). Synthetic fungicides can also leave toxic residues (Zahida and Masud, 2002).

These drawbacks of chemical control have increased interest in alternative control methods, particularly those that are environmentally safe (Conway *et al.,* 1991; Sugar *et al.,* 1997; Wilson *et at.,* 1997). Biological control is a possible alternative to fungicides in a postharvest environment, where temperature and relative humidity are controlled (Sports and Sanderson, 1994). Postharvest biocontrol is also a good idea because harvested fruits are easy to treat biologically. In banana crown rot, the pathogen often infects banana hands through wounds created during dehanding after harvest, indicating that colonization by antagonists is also a likely means of control.

Most approaches to the biological control of postharvest diseases have examined single biocontrol agents against diseases caused by single pathogens. Examples are, on banana, control of *C. musae* (anthracnose) with *Trichoderma viride* (Golam *et al.,* 1998), and, on yam, control of a postharvest disease caused by a complex of pathogens (*Aspergillus niger, L. theobromae* and *Penicillium oxalicum*) also using *T. viride* (Okigbo and Ikediugwa, 2000). The antagonistic potential of *Trichoderma* spp. against various postharvest pathogens has also been studied to counter green mould of citrus (De Matos, 1983), anthracnose of banana (Golam *et al.,* 1998), postharvest pathogens of rambutan (Sivakumar *et al.,* 2001), *Botrytis cinerea* on apple (Tronsmo, 1991) and fruit rot of strawberry (Tronsmo and Dennis, 1997).

Antagonists alone do not always give commercially acceptable control of fruit decay, but they can be enhanced by i) manipulation of the fruit surfaces ii) manipulation of the storage environment or iii) using combinations of beneficial organisms (Janisiewicz, 1996). In an ideal situation, one antagonistic organism should control all plant pathogens, but this is unrealistic, as biological control agents usually control only a narrow range of pathogens. Cook (1993) suggested that many antagonist strains may have to be formulated together to increase the range of control. Strain mixtures could mimic naturally occurring biological control (Janisiewicz, 1996; Raupauch and Klopper, 1998), to enhance the effectiveness and reliability of these strains (Duffy and Weller, 1995). Mixtures of antagonist strains usually produce greater biocontrol than the strains applied singly (Andrews, 1982). Mixed bioinocula have been pioneered to control soilborne diseases (Sivasithamparam and Parker, 1978; Raupauch and Kloepper, 1998; Malathi and Sabitha Doraisamy, 2004), and have also been applied in postharvest disease control (Janisiewicz, 1996; Leibinger *et al.,* 1997). An important prerequisite for strain mixtures is that the co-inoculated micro-organisms must be compatible (Baker, 1987; Krauss, *et al.,* 2001).

The objectives of the present study were first, to evaluate the effectiveness of both naturally occurring *Trichoderma* species from banana orchard soil and selected *Trichoderma* species from culture collections, against *L. theobromae* and C. *musae,* and second, to evaluate effective *Trichoderma* isolates singly and as mixtures for their control of crown rot incidence and banana shelf life, both at room temperature and under cold storage conditions, of bananas artificially inoculated with the two crown rot pathogens.

Materials and methods

Isolation of pathogens and preparation of pathogen inoculum

Lasiodiplodia theobromae and *Colletotrichum musae* were isolated from infected banana fruits and further purified by the hyphal tip method. The fungi isolated were identified by colony and conidium morphological characters according to Punithalingam (1976) and Sutton and Waterson (1970). Cultures of the two pathogens were maintained on potato dextrose agar (PDA) at 4°C. To produce conidia, mycelial discs (1.5 cm diam.) from two-week-old cultures of *L. theobromae* and *C. musae* were transferred to 100 mL of PDA in 250 mL conical flasks and incubated at 28±2°C for up to 10 days. After incubation, excess broth was drained, 30 ml of sterile water was added to each culture and the flasks were shaken at 50 rpm for 30 min on an orbital shaker. The content of each flask was then filtered through Whatman no. 2 filter paper to remove hyphal fragments. The resulting conidial suspension concentration of each

fungus was adjusted to 10 conidia mL^{-1} using a haemocytometer. These suspensions were used for the inoculation of banana hands in the experiments described below.

Isolation and collection of *Trichoderma* spp.

Trichoderma spp. were isolated from soil samples collected from five organic banana orchards (where leaf litter was allowed to accumulate) in the Thiruchirapalli district of Tamil Nadu, India. Soil suspensions were prepared by adding 1 g soil to 10 mL sterile distilled water and shaking for 15 min. Each suspension was then serially diluted up to 10^{-6}. From this dilution, a 0.1 mL aliquot was spread on a *Trichoderma* selective medium (TSM; Elad and Chet [1983]). Twelve *Trichoderma* isolates were obtained from the soil samples. These were identified as *T. viride* and *T. harzianum* by microscopic examination of the morphological and reproductive characters. These native *T. viride* isolates are hereafter identified as TV_1, TV_3, TV_4, TV_5, TV_6, TV_9, TV_{10} and TV_{11} and the native *T. harzianum* isolates as TH_{11}, TH_{12}, TH_{13} and TH_{14}. A further eight isolates of *Trichoderma* were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, India. These were the introduced isolates and were identified as *T. viride* 1, *T. harzianum* 1, *T. harzianum* 2, *T. harzianum* 3, *T. reseei, T. pseudokoningii, T. koningii* and *T. virens*. All 20 *Trichoderma* spp. were maintained on PDA at 4°C.

Examination of the antagonism of *Trichoderma* spp.

Dual culture assay

The effectiveness of the native and introduced *Trichoderma* isolates against mycelial growth of *L. theobromae* and *C. musae* was tested by a dual culture technique (Dennis and Webster, 1971). For each test, an 8 mm diam. mycelial disc from a 7-day-old culture of *L. theobromae* or C. *musae* was placed on the agar surface of a 90 mm Petri dish 1 cm from the edge of the dish. An 8 mm diameter mycelial disc from an actively growing *Trichoderma* sp. culture was then placed on the agar surface opposite the target pathogen. Four replicate plates were maintained for each treatment. The plates were incubated together with the experimental controls (pathogen without *Trichoderma*) at 28±2°C for 4 days, and the radial growth (mm) of the pathogen mycelium was recorded. Per cent inhibition of mycelial growth of the pathogen was calculated.

Conidial germination assay

Of the 20 *Trichoderma* isolates tested by dual culture, eight (TV_3, TV_4, TV_9, TV_{11}, *T. harzianum* 1, *T. harzianum* 3, *T. koningii* and *T. pseudokoningii*) showed some activity. Culture filtrates of these isolates were tested against the conidial germination and the germ tube elongation of the test pathogens. The eight isolates were grown separately for l week at 28±2°C in 250 mL conical flasks containing potato dextrose broth. The fungal cells were then removed from each culture by filtering first through two layers of muslin cloth and then through a 0.2 μm Millipore filter. One mL of filtrate from each isolate was placed on a cavity microscope slide. Then 1 ml of a conidial suspension (10^6 conidia mL^{-1}) of *L. theobromae* or *C. musae* in sterile distilled water was added to the slide and mixed thoroughly. The slides were kept in Petri dishes on a glass bridge chamber and incubated at 25°C. Conidial suspensions of *L. theobromae* or *C. musae* in sterile distilled water served as the controls. Conidial germination of the pathogens was assessed for up to 4 days at 4 h intervals, and per cent germination of the conidia was calculated by scoring 100 conidia for germination. The experiments were replicated four times to obtain an average per cent germination.

Fruit preparation

Bananas cv. Robusta (AAA group) were harvested at 75–80% maturity from a commercial plantation in Thiruchirapalli district, Tamil Nadu, India. Banana hands free of visual defects with uniform shape and weight were selected for the experiment. The hands were thoroughly washed in running tap water to remove dust, then surface-sterilized with 70% ethanol and allowed to dry for 6 h at room temperature. Treatments were applied within 24 h of harvesting.

Effect of *Trichoderma* spp. on crown rot of banana

The most effective *Trichoderma* spp. screened *in vitro* against *L. theobromae* and *C. musae* were

evaluated singly and in various combinations in two tests to determine their activity against crown rot and their capacity to extend the shelf life of banana fruit. For each test, a small cavity was made with a sterile needle on the cut surface of a banana hand. This cavity was inoculated with 500 μL of a conidial suspension of *L. theobromae* and C. *musae* (10^6 conidia mL^{-1}) and kept for 2 h at 28±2°C. In a first set of experiments, the hands were sprayed separately with conidial suspensions of eight *Trichoderma* spp. isolates (10^9 conidia mL^{-1}) which had been effective against the pathogens in the *in vitro* assays. After spraying with *Trichoderma*, the fruits were air-dried and each hand was loosely packed in a polythene bag which was closely tied at the top. Two sets of identical experiments were carried out, one at room temperature (28±2°C) and the other under cold storage conditions (14°C, 90% RH). Each treatment was replicated four times. The banana hands were scored for severity of crown rot (0–5 scale), crown colour (1–7 scale) and crown texture (0–4 scale) (Finlay and Brown, 1993), and the shelf life was determined.

A second set of crown rot experiments was carried out using the same methods to test pair-wise combinations of *Trichoderma* isolates that had been effective on the fruits when applied singly. The isolates selected were TV_4, TV_9, *T. harzianum* 3 and *T. koningii*. All possible pairings of these isolates were made in such a way as to include the *Trichoderma* isolates that were most effective against *L. theobromae* and *C. musae*. The 11 possible pairing of the antagonists (TV_4+ TV_9; TV_9+*T. harzianum* 3; TV_9+*T. koningii;* TV_4+*T. harzianum* 3; TV_4+*T. koningii*; *T. harzianum* 3+*T. koningii*; TV_4+TV_9+*T. harzianum* 3; TV_4+TV_9+*T. koningii*; TV_4+*T. harzianum* 3+*T. koningii*; TV_9+*T. harzianum* 3+*T. koningii* 3; TV_4+TV_9+*T. harzianum* 3+*T. koningii)* were evaluated on the fruits. The controls were not inoculated. After spraying with the *Trichoderma* suspensions, fruits were air dried and placed in polythene bags. Two experiments were carried out; one at room temperature (28±2°C) and the other under cold storage (14°C, 90% RH). Each treatment was replicated four times and scored as above.

Data analysis

All the experiments were of completely randomized design (CRD) and repeated twice. To determine the effect of each *Trichoderma* sp. on the radial growth and conidial germination of *L. theobromae* and *C. musae*, the per cent reduction compared with the experimental controls was calculated. The data were analyzed using the IRRISTAT version 92-1 program developed by the biometrics unit, International Rice Research Institute, Manila, the Philippines. Data were subjected to analysis of variance (ANOVA). The treatment means were compared with Duncan's multiple range test (DMRT) at the 5% significance level (Gomez and Gomez, 1984).

Results

Effects of native and introduced *Trichoderma* spp. on *in vitro* mycelial growth of *L. theobromae* and *C. musae*

Of the 12 native isolates of *Trichoderma* spp. tested for their effectiveness against mycelial growth, TV_9 exhibited maximum inhibition of *L. theobromae* compared with the controls (91.7%), followed by TV_{11} (88.7%) and TV_6 (84.5%). For *C. musae*, maximum inhibition was achieved with TV_3 and TV_4 (both 86.3%) followed by TH_{14} (79.6%) (Table 1). All eight introduced *Trichoderma* spp. inhibited mycelial growth of *L. theobromae,* with reductions ranging from 45.1 to 85.0% (Table 2). Of these, *T. harzianum* 3 most strongly inhibited *L. theobromae* (85.0%) followed by *T. koningii* (76.7%). Of the eight introduced antagonists tested on *C. musae, T. harzianum* 1 and *T. pseudokoningii* had the greatest inhibition (both 87.5%) followed by *T. harzianum* 2 (82.2%).

Effects of native and introduced *Trichoderma* spp. on conidial germination of *L. theobromae* and *C. musae*

The culture filtrate of TV_9 reduced conidial germination of *L. theobromae* to 22.3% compared with 85.2% in the controls. Germ tube length was also greatly reduced by the culture filtrate of isolate TV_9 compared with the other isolates. Isolates *T. harzianum* 3 and TV_3 reduced both conidial germination and germ tube length. Of the culture filtrates tested on *C. musae,* isolate TV_4 and *T. koningii* reduced conidial germination to 19.7 and 25.2% compared with 82.8% in the controls, and both isolates also reduced germ tube length more than did other isolates (Table 3).

Table 1. Mean mycelium growth of *Lasiodiplodia theobromae* and *Colletotrichum musae* and proportional reductions (compared with controls) for native *Trichoderma* isolates tested *in vitro*.

Trichoderma isolate [a]	*L. theobromae*		*C. musae*	
	Mycelial growth [b, c] (mm)	Reduction over control (%)	Mycelial growth [b, c] (mm)	Reduction over control (%)
TV_1	27.7 f	68.7	20.7c	74.3
TV_3	27.7 f	68.7	10.3 a	87.1
TV_4	26.0 e	70.6	11.0 a	86.3
TV_5	23.3 d	73.6	32.0 e	60.2
TV_6	13.7 c	84.5	28.0 d	65.1
TV_9	07.3 a	91.7	36.3 f	54.8
TV_{10}	42.3 h	52.1	41.3 g	48.5
TV_{11}	10.0 b	88.7	43.0 h	46.5
TH_{11}	37.0 g	58.1	32.3 e	59.7
TH_{12}	45.3 i	48.7	42.3 gh	47.3
TH_{13}	38.0 g	60.0	43.3 h	46.0
TH_{14}	44.0 i	50.2	16.3 b	79.7
Control	88.3 j	-	80.3 i	-

[a] TV, *Trichoderma viride*, TH, *Trichoderma harzianum*.
[b] Values are the means of four replications.
[c] In a column means followed by a common letter are not different ($P<0.05$) at 5 per cent level, indicated by DMRT.

Table 2. Mean mycelium growth of *Lasiodiplodia theobromae* and *Colletotrichum musae* and proportional reductions (compared with controls) for different introduced *Trichoderma* isolates tested *in vitro*.

Trichoderma sp.	*L. theobromae*		*C. musae*	
	Mycelial growth [a, b] (mm)	Reduction over control (%)	Mycelial growth [a, b] (mm)	Reduction over control (%)
T. viride 01	27.3 d	69.2	25.7 c	70.9
T. harzianum 1	48.7 f	56.8	11.0 a	87.5
T. harzianum 2	25.3 c	71.5	15.7 b	82.2
T. harzianum 3	13.3 a	85.0	35.7 e	59.6
T. *reseei*	32.3 e	63.6	36.0 e	59.2
T. *pseudokoningii*	46.3 f	47.8	11.0 a	87.5
T. *koningii*	20.7 b	76.7	28.7 d	67.5
T. *virens*	48.9 f	45.1	37.3 f	57.7
Control	88.7 g	-	88.3 g	-

[a] Values are the means of four replications.
[b] In a column means followed by a common letter are not different ($P<0.05$) at 5 per cent level, indicated by DMRT.

Table 3. Mean percent germination and germ tube length of conidia of *Lasiodiplodia theobromae* and *Colletotrichum musae* sprayed with culture filtrates of different *Trichoderma* spp. isolates.

Culture filtrate (40%)[a]	*L. theobromae* [b, c]			*C. musae* [b, c]		
	Germination (%)		Germ tube length (μm)	Germination (%)		Germ tube length (μm)
TV_3	31.5 b	(34.1)	284.3 c	28.5 bc	(32.3)	172.3 b
TV_4	43.2 c	(41.1)	393.3 f	19.7 a	(26.3)	122.7 a
TV_9	22.3 a	(28.2)	85.3 a	29.2 c	(32.7)	168.8 b
TV_{11}	42.6 c	(40.7)	395.7 f	41.8 e	(40.3)	193.7 d
Trichoderma harzianum 1	31.5 b	(34.1)	305.0 d	35.8 d	(36.8)	184.0 c
T. harzianum 3	32.2 b	(34.6)	259.7 b	31.5 c	(34.1)	308.3 g
T. koningii	31.5 b	(34.1)	378.7 e	25.2 b	(30.1)	291.7 f
T. pseudokoningii	43.5 c	(41.3)	378.0 e	45.6 e	(42.5)	264.7 e
Control	85.2 d	(67.5)	717.3 g	82.8 f	(65.6)	700.0 h

[a,b,c] See Table 1.

Effectiveness of *Trichoderma* spp. in the control of crown rot and the effects of *Trichoderma* on the shelf life of banana

The crown rot incidence of all antagonist-sprayed fruits both at room temperature and in cold storage was less than that of the control fruit ($P<0.05$) (Table 4). However, of the eight most promising antagonists (selected *in vitro*), tested on the fruits, isolates TV_4, TV_9 and *T. harzianum* 3 achieved crown rot severity scores of less than 2.0, compared with a severity score of 4.0 in pathogen inoculated hands. Other isolates were less effective but they still decreased crown rot. The colour and texture scores of the crowns were also reduced in fruit sprayed with three isolates as compared with the controls. The three isolates were less effective than carbendazim in reducing crown rot severity.

The assessment of single antagonist sprays to enhance the shelf life of bananas, both at room temperature (28°C) and in cold storage, indicated that these antagonists enhanced total shelf life (green and yellow period) ($P<0.05$) of the fruit to 10 to 14 days at room temperature and 30 to 45 days at cold storage, compared with pathogen-inoculated hands. Single antagonists did not extend shelf life as much as did carbendazim (0.1%). Maximum shelf life was achieved with carbendazim (16 days at 28°C and 62 days at 14°C), TV4 and *T. harzianum* 3. In *L. theobromae* and *C. musae* inoculated hands, total shelf life was 8 days at room temperature and 28 days in cold storage.

Effects of antagonist mixtures for controlling crown rot and effects on shelf life of banana

The isolates of *Trichoderma* spp. that were promising when tested singly were further tested in all possible combinations as inoculum mixtures. When isolates were paired with one another on water agar, no antagonism between them was observed (data not shown). Of the 11 possible combinations of mixed inocula tested on banana fruits, a clear pattern emerged: the higher the number of strains in a mixture, the lower was the level of crown rot severity and the greater the shelf life of the fruit. The mixtures containing four isolates gave 100 per cent reduction of crown rot severity for up to 12 days of incubation. The next most effective treatments were the three-isolate mixtures, such as TV_4+TV_9+*T. harzianum* 3; TV_9+*T. harzianum* 3+*T. koningii;* and TV_4+TV_9+T. *koningii*. All these mixtures gave a crown rot score of 1 at room temperature. The scores for crown colour and texture were also lower in fruits

Table 4. Mean crown rot severity, colour and texture scores, and mean shelf life, for banana fruit cv. Robusta sprayed with different *Trichoderma* spp. isolates.

Treatment[a]	Severity score after 10 d at 28°C [b, c]				Severity score after 25 d at 14°C [b, c]			
	Rot (0–5)	Colour (1–7)	Texture (0–4)	Shelf life (d)	Rot (0–5)	Colour (1–7)	Texture (0–4)	Shelf life (d)
TV_3	2.0 bcd	5.8 b	2.3 d	11.3 d	1.7 cd	4.8 a	1.7 bc	35.7 f
TV_4	1.7 bc	4.5 a	1.3 bc	13.7 bc	1.3 bc	5.8 bc	1.7 bc	43.3 de
TV_9	1.3 b	5.0 a	1.8 bcd	14.0 b	1.3 bc	4.8 a	2.0 bc	45.0 c
TV_{11}	2.3 cd	5.8 b	2.0 cd	11.7 d	1.7 cd	5.8 bc	2.0 bc	36.0 f
Trichoderma harzianum 1	2.3 cd	5.8 b	1.3 abc	10.3 e	2.0 cd	4.8 a	1.7 bc	32.3 g
T. harzianum 3	1.7 bc	4.8 a	1.0 ab	14.3 b	1.3 bc	4.5 a	1.3 ab	44.0 d
T. koningii	2.0 bcd	5.0 a	2.0 cd	13.0 c	1.7 cd	5.8 bc	1.7 bc	43.0 e
T. pseudokoningii	2.7 d	5.0 a	2.3 d	10.3 e	2.3 d	6.3 cd	2.3 c	30.0 h
Control (no pathogen)	0.3 a	4.8 a	1.0 ab	16.0 a	0.7 ab	5.5 b	0.7 a	55.3 b
L. theobromae+ *C. musae*	4.0 e	6.5 c	3.8 e	8.0 f	4.3 e	6.5 d	4.0 d	26.3 i
Carbendazim (0.1%)	0. 3 a	4.5 a	0.8 a	16.3 a	0.0 a	4.8 a	0.8 a	62.0 a

[a,b,c] See Table 1.

treated with the four-way and all the three-way mixtures compared with the experimental controls ($P<0.05$). The four-way isolate mixture reduced crown rot severity to a level equivalent to that achieved with carbendazim.

With fruit incubated at cold storage (14°C) the mixture of four antagonists (TV_4+TV_9+*T. harzianum* 3+*T. koningii*) gave a crown rot severity score of 0 after 25 days of incubation, a colour score of 1.0 and a texture score of 0. The three-way isolate mixtures (TV_4+TV_9+*T. harzianum* 3; TV_9+*T. harzianum* 3+*T. koningii;* TV_4+*T. harzianum* 3+*T. koningii*) also reduced the crown rot score to less than 1.0. In cold storage, the effectiveness of the four-way mixture for reducing crown rot severity was comparable to that achieved with carbendazim (0.1%) at 25 days of incubation (Table 5).

All the two-way, three-way and four-way mixtures increased ($P<0.05$) the banana shelf life to 11–16 days at room temperature and to 45–62 days at cold storage. The increase in shelf life was greatest in fruit sprayed with the four-way isolate mixture (17 days at 28°C and 62 days at 14°C), and this was not different ($P<0.05$) from the increase with carbendazim. The three-way isolate mixture also increased fruit shelf life up to 58–60 days. The shelf life achieved with the two-way mixtures was less than that achieved with the three- or four-way mixtures.

Discussion

Crown rot is a very serious problem with commercial bananas, especially in the Cavendish varieties with an AAA genome. It is often difficult

Table 5. Mean crown rot severity, colour and texture scores, and mean shelf life, for banana fruit cv. Robusta sprayed with different mixtures of *Trichoderma* spp. isolates.

Treatment [a]	Severity score after 12 d at 30°C [b, c]				Severity score after 25 d at 14°C [b, c]			
	Rot (0–5)	Colour (1–7)	Texture (0–4)	Shelf life (d)	Rot (0–5)	Colour (1–7)	Texture (0–4)	Shelf life (d)
TV_4+TV_9	1.0 bc	5.0 b	0.7 b	15.7 abc	0.7 ab	5.0 ab	0.7 bc	54.3 ef
TV_4+*T. harzianum* 3	1.0 bc	5.7 c	0.7 b	15.0 cd	0.7 ab	4.7 ab	0.7 bc	53.3 g
TV_9+*T. koningii*	1.7 c	6.0 cd	2.0 c	12.0 f	1.7 c	5.3 b	1.3 d	45.3 h
TV_4+*T. koningii*	1.7 c	6.3 d	2.0 c	11.3 f	1.7 c	5.3 b	1.3 d	45.7 h
TV_9+*T. harzianum* 3	1.0 bc	5.7 c	0.7 b	14.0 e	0.3 ab	4.3 a	0.3 ab	55.7 d
T. harzianum 3+*T. koningii*	0.7 ab	5.0 b	0.7 b	14.3 de	0.7 ab	5.0 ab	0.3 ab	55.0 de
TV_4+TV_9+*T. harzianum* 3	0.7 ab	5.0 b	0.7 b	15.0 cd	0.7 ab	4.7 ab	0.3 ab	58.3 c
TV_4+TV_9+*T. koningii*	0.7 ab	5.0 b	0.7 b	15.3 bc	0.3 ab	5.0 ab	0.3 ab	58.3 c
TV_4+*T. harzianum* 3 +*T. koningii*	0.3 ab	4.7 b	0.0 a	15.3 bc	0.3 ab	4.7 ab	0.3 ab	58.0 c
TV_9+*T. harzianum* 3 +*T. koningii*	0.3 ab	4.0 a	0.0 a	16.0 ab	0.3 ab	4.7 ab	0.0 a	59.7 b
TV_4 +TV_9+*T. harzianum* 3 +*T. koningii*	0.0 a	4.0 a	0.0 a	16.3 a	0.0 a	4.7 ab	0.0 a	62.0 a
Control (no pathogen)	1.0 bc	5.0 b	0.7 b	16.0 ab	1.0 bc	5.0 ab	1.0 cd	54.0 fg
L. theobromae+*C. musae*	4.3 d	7.0 e	3.7 d	8.3 g	4.3 d	6.3 c	3.7 e	27.3 i

[a,b,c] See Table 1.

to identify individual biocontrol agents that control diseases caused by pathogen complexes. Suitable antagonistic micro-organisms could however replace the synthetic fungicides. In the present study 12 native *Trichoderma* species isolated from banana plantation soils, and eight species from a culture collection were tested *in vitro* against the two banana crown rot pathogens. The native isolates TV_9 and TV_{11} and the introduced isolates *T. harzianum* 3 and *T. koningii* were antagonistic to *L. theobromae* and the native TV_3 and TV_4 and the introduced *T. harzianum* 1 and *T. pseudokomngii* were antagonistic to *C. musae*. Microscopic examination of the interactions of *T. viride* and *T. harzianum* isolates with *L. theobromae* and *C. musae* revealed direct parasitism by the antagonists through appressed growth and coiling around the mycelium of the pathogens.

Trichoderma spp. and isolates clearly exhibited varying levels of antagonism towards *L. theobromae* and *C. musae*. A specificity of biocontrol agents against the pathogens was also evident. On both pathogens, the antagonists isolated from the soils of organic banana orchards performed better than the antagonists from the culture collection. This is consistent with Smolka (1992), who reported that a pool of antagonists obtained from natural vegetation is likely to provide better control of pathogens. Several reports have examined the effectiveness of native antagonists against postharvest pathogens of different fruit crops (Kanapathipillai and

Jantan, 1986; Borras and Aguilar, 1990; Huang *et al.,* 1992; Arras, 1993; Testoni *et al.,* 1993). Native antagonists were found to be effective in fruits such as apple (Janisiewiz *et al.,* 1991), avocado (Korsten *et al.,* 1994), banana (Costa and De Subasinghe, 1998), and mango (Koomen and Jeffries, 1993). In our study the introduced antagonists *T. harzianum* 1, *T. harzianum* 3 and *T. koningii* also performed well but with varying levels of specificity against both the test pathogens. Bhuvaneswari and Rao (2001) evaluated postharvest pathogens of mango. They found that the growth of *Pestalotia* sp., *Aspergillus flavus, L. theobromae, C. gloeosporioides, Rhizopus stolonifer* and *A. niger* was inhibited in varying degrees by antagonist fungi.

In the present study, the culture filtrates of TV_9 and *T. harzianum* 3 had the greatest effect on conidial germination and germ tube growth of *L. theobromae,* and the culture filtrates of TV_4 and *T. koningii* had the greatest effect on the conidia of *C. musae.* The inhibitory effect of the culture filtrates of *Trichoderma* spp. on both pathogens may be due to the production of metabolites, which has been reported by Horvath *et al.* (1998), Ghisalberti and Rowland (1993) and Iqbal *et al.* (1994). Furthermore, when eight isolates that reduced pathogen growth *in vitro* were tested for their effectiveness against crown rot of the fruits, these isolates were effective both at room temperature (28°C) and in cold storage (14°C). Of the isolates, the native strains such as TV_4 and TV_9 and the introduced isolates *T. harzianum* 3 and *T. koningii* reduced crown rot score more than the other isolates, but not as much as a fungicide. Sivakumar *et al.* (2001) reported that six native isolates of *T. harzianum* obtained from rhizosphere soil were effective against the rambutan postharvest pathogens *B. theobromae, C. gloeosporioides* and *Gliocephalotrichum microchlamydosporum*, and did not affect the overall quality and colour of the fruit.

The results imply that the complex of banana crown rot pathogens may lead to specific genus discrimination by the antagonists, resulting in reduced biocontrol of the disease. Hence, single antagonist isolates may not achieve effective biological control in the long run. These findings are consistent with those of Baker (1991) and Janisiewicz (1996), who reported that micro-organisms used as biocontrol agents typically had a relatively narrow spectrum of action. Similarly, Krauss *et al.* (2001) reported that when antagonists were used to control *C. musae* in banana, the existence of different strains (significant biodiversity) of the pathogen led to strain discrimination by the antagonists, giving inconsistent and variable biocontrol.

Fukui *et al.* (1999) suggested that the best practical approach is to start the evaluation of biocontrol agents with mixtures of many antagonists and then eliminate the ineffective or incompatible ones. In the present study, consortia of four isolates effectively reduced crown rot incidence up to 25 days. Three-way mixtures were also effective, but less so than the four-way mixtures. The four-way isolate mixture were equally effective as carbendazim, both at room temperature and in cold storage. Total shelf life was also maximized with the four-way isolate mixtures (62 days), as long as that with carbendazim. An effective four-way mixture consisted of both native and introduced *Trichoderma* spp. and included isolates effective against both *L. theobromae* and *C. musae*. Similarly, Leibinger *et al.* (1997) reported that a combination of two strains of *Aureobasidium pullans* and the yeast *Rhodotorula glutinans,* when applied simultaneously, controlled *Botrytis cinerea, Penicillium expansum* and *Pezicula malicorticis* as effectively as a commercial fungicide. Mixed bioinocula of antagonists have been successful against several pathogens infecting fruit crops (Schisler *et al.,* 1997; Guetsky *et al.,* 2001).

Isolate mixtures are more likely to mimic naturally occurring biological control, which involves mixed antagonist populations rather than large populations of single antagonists (Raupach and Klopper, 1998). With results similar to ours, Krauss *et al.* (2001) demonstrated that mixtures of up to four fungal antagonists were increasingly effective in controlling mixed infection of banana crown rot caused by four strains of C. *musae*. Antagonist mixtures may also help to achieve useful levels of biocontrol protection under fluctuating environmental condition, where different antagonistic mechanisms may be inactivatead by particular environmental conditions (Sivasithamparam and Parker, 1978; Pierson and Weller, 1994).

Contrary findings have however also been

recorded. Combinations of antagonists that did not enhance disease protection as compared with the antagonists applied singly were reported by Sneh, *et al*. (1984) and Leibinger *et al*. (1997). In the present study, effective isolate mixtures included both native and introduced strains, and no antagonism between them was detected in water agar cultures. A biocontrol product consisting of a mixture of strains will have a potential drawback in that producing and registering such a product is likely to be more costy and difficult than to produce and register a product consisting of only a single antagonist strain. In addition, the synergism of biocontrol activity is more likely with selected combinations of antagonists (Schisler *et al.,* 1997). There is an urgent need to find alternative control methods for postharvest diseases that do not rely on fungicide chemicals, since pathogens build up resistance to these chemicals, and since there are increasing concerns about the effect of pesticides on human health. Further research is required before the biocontrol application reported here can be used commercially. Future studies will therefore be aimed at developing suitable formulations for antagonist mixtures to be used on a large scale in postharvest conditions.

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